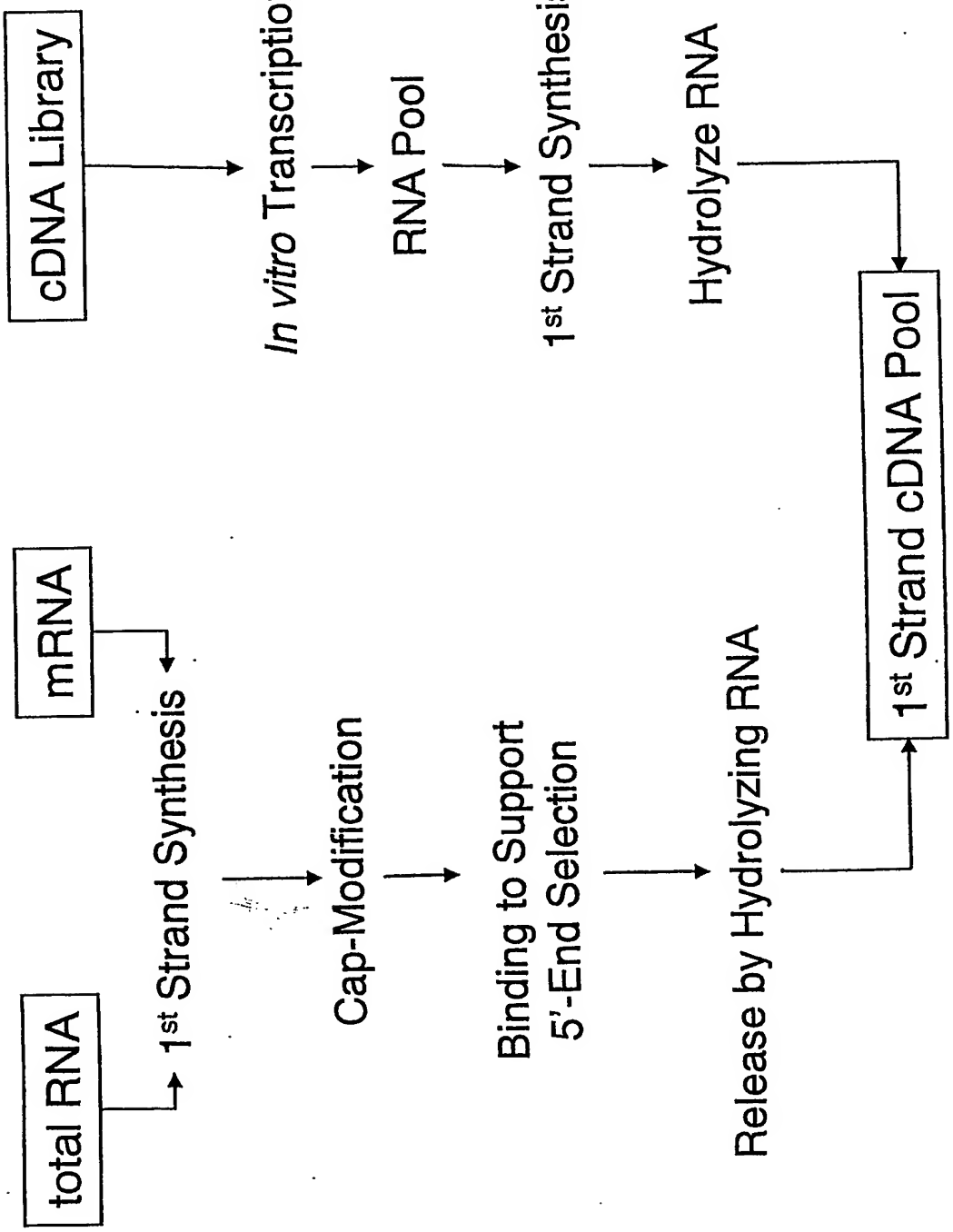
cDNA Library
In vitro Transcription
RNA Pool
1st Strand Synthesis
Hydrolyze RNA
1st Strand cDNA Pool
mRNA
1st Strand Synthesis
Cap-Modification
Binding to Support
5'-End Selection
Release by Hydrolyzing RNA
total RNA

Fig.1

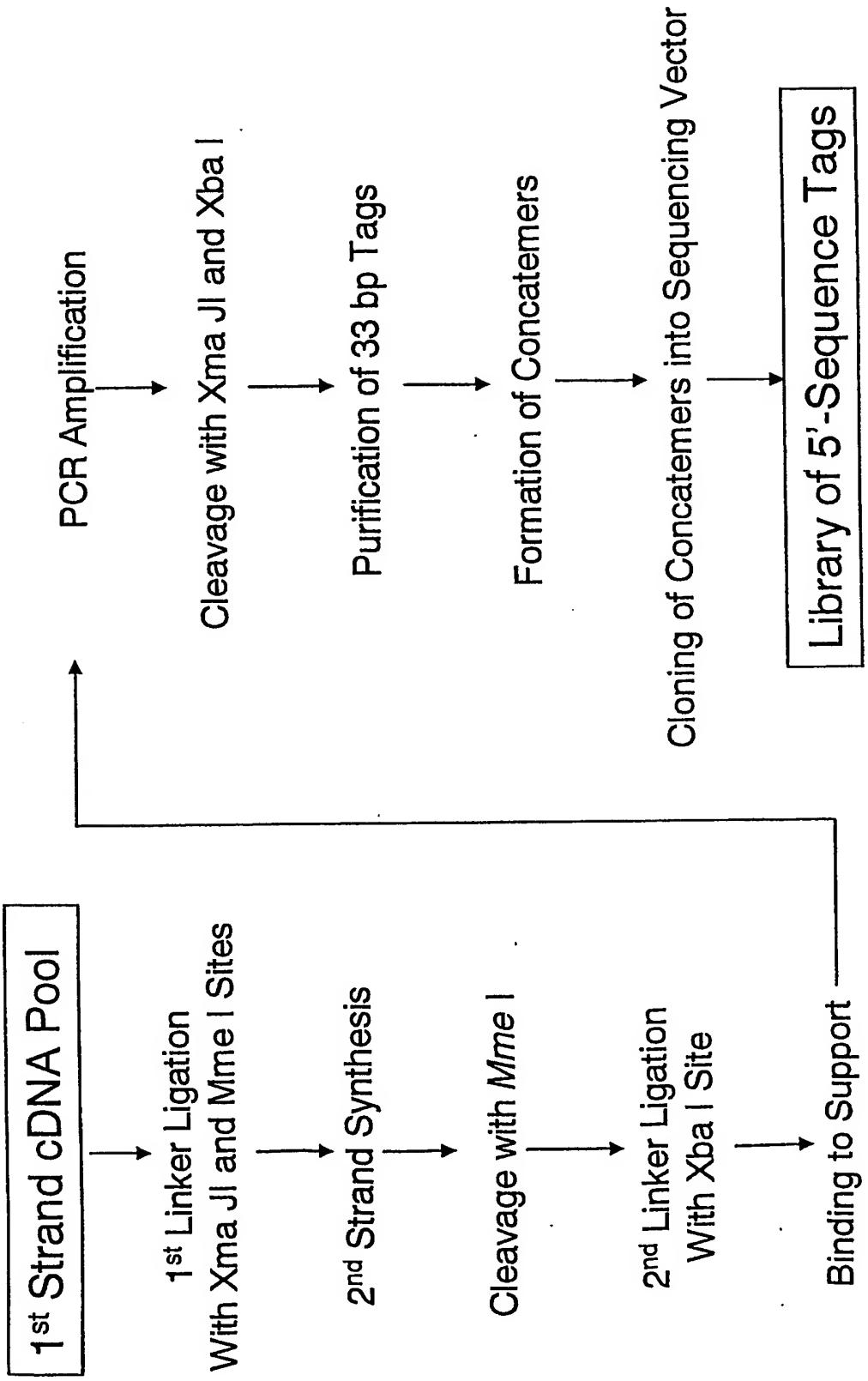
1st Strand cDNA Pool
1st Linker Ligation With Xma JI and Mme I Sites
2nd Strand Synthesis
Cleavage with Mme I
2nd Linker Ligation With Xba I Site
Binding to Support
PCR Amplification
Cleavage with Xma JI and Xba I
Purification of 33 bp Tags
Formation of Concatemers
Cloning of Concatemers into Sequencing Vector
Library of 5'-Sequence Tags

Fig.2

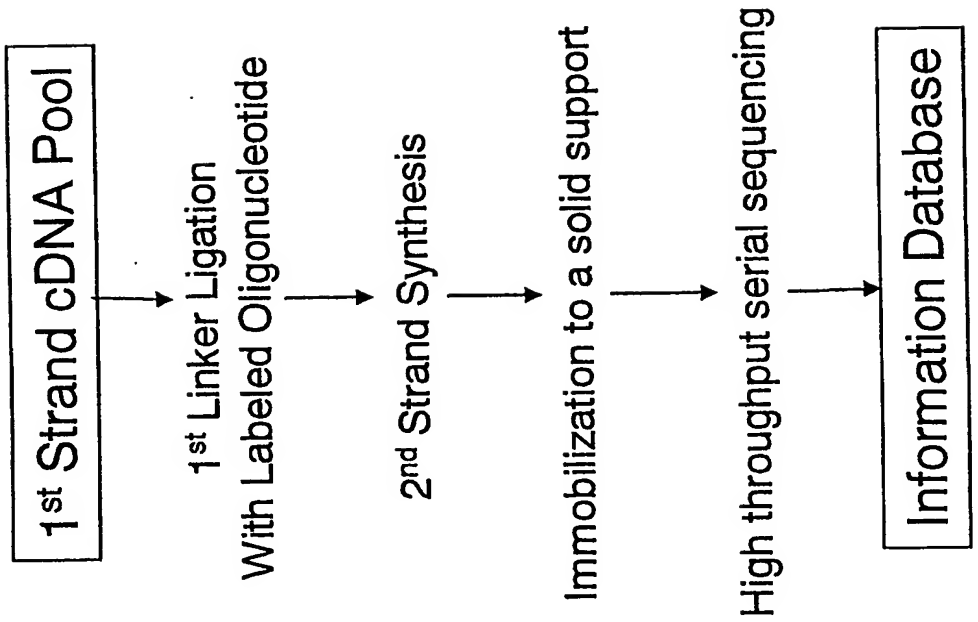
1st Strand cDNA Pool
1st Linker Ligation
With Labeled Oligonucleotide
2nd Strand Synthesis
Immobilization to a solid support
High throughput serial sequencing
Information Database

Fig. 3

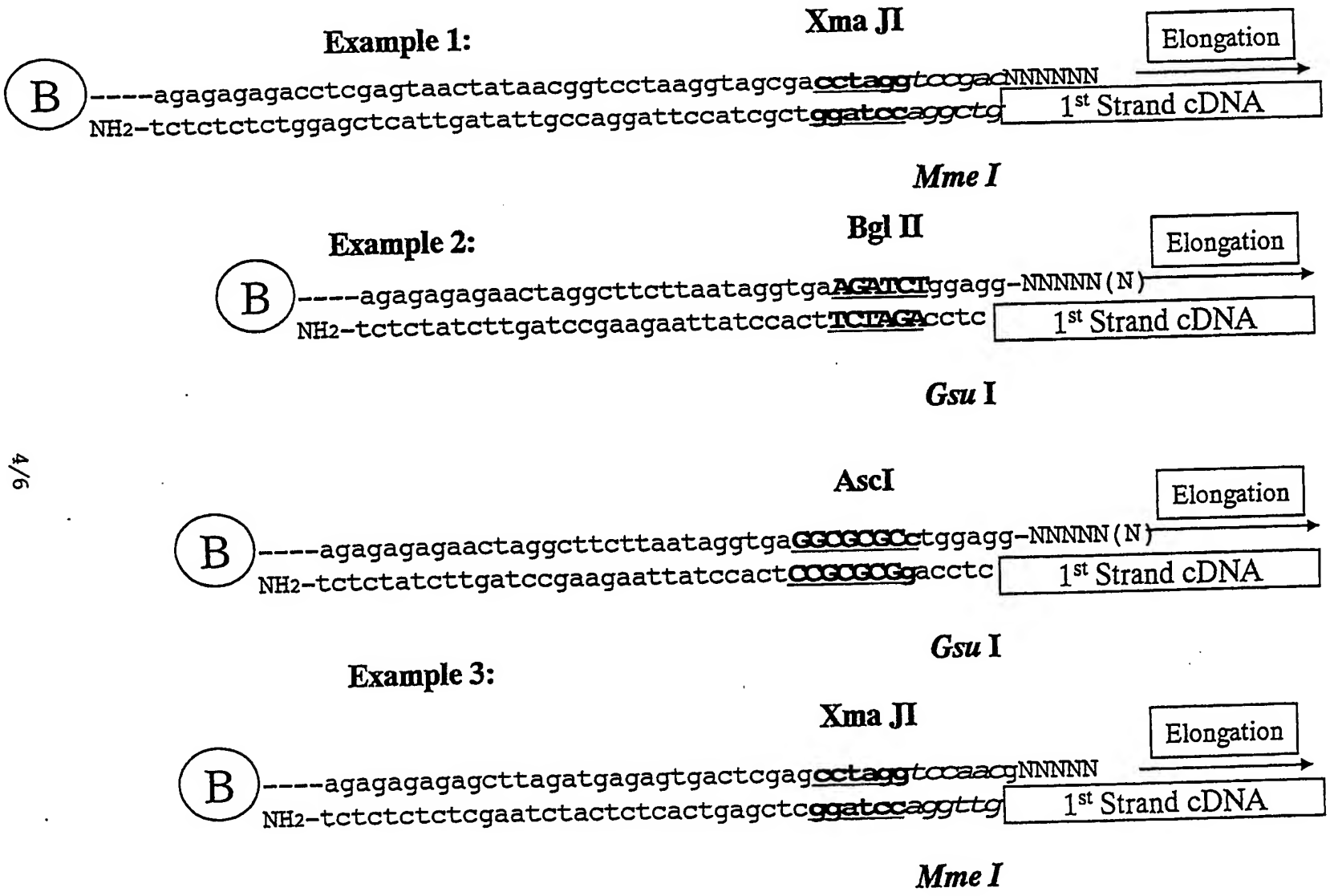
Example 1:
Xma JI
Elongation
B
-----agagagagacctcgagtaactataacggtcctaaggtagcgacctaggtccgacNNNNNN
NH2-tctctctctggagctcattgatattgccaggattccatcgctggatccaggctg
1st Strand cDNA
Mme I
Example 2:
Bgl II
Elongation
B
-----agagagagaactaggcttcttaataggtgaAGATCTggagg-NNNNN(N)
NH2-tctctatcttgatccgaagaattatccactTCTAGAcctc
1st Strand cDNA
Gsu I
AscI
Elongation
B
-----agagagagaactaggcttcttaataggtgaGGCGCGCctggagg-NNNNN(N)
NH2-tctctatcttgatccgaagaattatccactCCGCGCGgacctc
1st Strand cDNA
Gsu I
Example 3:
Xma JI
Elongation
B
-----agagagagagcttagatgagagtgactcgagcctaggtccaacgNNNNNN
NH2-tctctctctcgaatctactctcactgagctcggatccaggttg
1st Strand cDNA
Mme I

Fig.4

Fig.5

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Example 1:

...tctaga NNNNNNNNNNNNNNNNNNN gtcggacctaggtccgac NNNNNNNNNNNNNNNNNNN tctaga..
...agatct NNNNNNNNNNNNNNNNNNN cagcctggatccaggctg NNNNNNNNNNNNNNNNNNN agatct..

Xba I Xma JI Xba I

...tctaga NNNNNNNNNNNNNNNNNNN gtcgga cctaga NNNNNNNNNNNNNNNNNNN gtcgga cctaga
...agatct NNNNNNNNNNNNNNNNNNN cagcct ggatct NNNNNNNNNNNNNNNNNNN cagcct ggatct

Xba I Xma JI/Xba I Xma JI/Xba I

Example 2:

...gaattc NNNNNNNNNNNNNNNNN ctccAGATCTggag NNNNNNNNNNNNNNN gaattc ...
...cttaag NNNNNNNNNNNNNNNNN gaggTCTAGAcctc NNNNNNNNNNNNNNN cttaag ...

Eco RI Bgl II Eco RI

...gaattc NNNNNNNNNNNNNNNNN cctccaGGCGCGCCtggagg NNNNNNNNNNNNNNN gaattc..
...cttaag NNNNNNNNNNNNNNNNN ggaggtCCGCGCGGacctcc NNNNNNNNNNNNNNN cttaag ...

Eco RI AscI Eco RI

Example 3:

... gaattc NNNNNNNNNNNNNNNNNNN gttggacctaggtccaac NNNNNNNNNNNNNNNNNNN gaattc..
... cttaag NNNNNNNNNNNNNNNNNNN caacctggatccaggttg NNNNNNNNNNNNNNNNNNN cttaag..

Eco RI Xma JI Eco RI

Fig.6

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PCT/JP03/07514